NITROGEN FIXING ENDOPHYTIC MICROBES FROM CEREAL CROPS AND THEIR BIOTECHNOLOGICAL APPLICATIONS

SYNOPSIS

Submitted to Eternal University

In the partial fulfillment

For the Degree of

Doctor of Philosophy in Biotechnology

By

Kusam Lata Rana (BS15PSBT003)

Under the Supervision

of

Dr. Ajar Nath Yadav

Assistant Professor

Department of Biotechnology
Akal College of Agriculture
Eternal University, Baru Sahib
Himachal Pradesh -173101
2016

SYNOPSIS

Name of the Student	:	KUSAM LATA RANA
Registration No	:	BS15PSBT003
Major Subject	:	Biotechnology
Major Advisor	:	Dr. Ajar Nath Yadav (Assistant Professor)
Co-Major Advisor	:	Dr. Vinod Kumar (Assistant Professor)
Name of Department	:	Biotechnology
Name of college	:	Akal College of Agriculture

Title: Nitrogen Fixing Endophytic Microbes from Cereal Crops and their Biotechnological Applications

I. INTRODUCTION

The endophytic microbes are referred as to those microorganisms, which colonize in the interior of the plant parts, *viz:* root, stem or seeds without causing any harmful effect on host plant. The word *endophyte* means 'in the plant' and is derived from the Greek words *endon* (within) and *phyton* (plant). The usage of this term is as broad as its literal definition and spectrum of potential hosts and inhabitants, e.g. bacteria (Kobayashi and Palumbo 2000), fungi (Stone et al 2000), plants (Marler et al 1999) and insects of plants (Feller 1995). Endophytes have been defined by various authors in somewhat different ways (Schulz and Boyle 2005, Rosenblueth and Martínez-Romero 2006, Mercado-Blanco and Lugtenberg 2014). Microbial endophytes can be isolated from surface-disinfected plant tissue or extracted from internal plant tissue (Hallmann et al 1997). Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant. These microorganisms can reside within cells (Jacobs et al 1985), in the intercellular spaces (Patriquin and Döbereiner 1978) or in the vascular system (Bell et al 1995). Endophytic microbes enter plants mainly through wounds, naturally occurring as a result of plant growth or through root hairs and at epidermal conjunctions. Besides providing entry avenues, wounds also create favorable conditions for the bacteria by allowing leakage of plant exudates that serve as a nutrient source for the bacteria (Quadt-Hallmann et al 1997). Other entry sites for endophytic microbes include flowers, stomata and lenticels (Kluepfel 1993). Once in the plant, the endophytic microbes have an ecological advantage over the epiphytic microbes in that they are protected from adverse external environmental conditions such as those associated with

temperature, salinity, drought, pH, osmotic potentials, and ultraviolet radiation, which are major factors limiting long-term microbial survival. The population density of endophytic microbes is highly variable, depending mainly on the microbial species and host genotypes but also on the host developmental stage, inoculum density, and environmental conditions. Molecular analysis has shown that plant defense responses also limit bacterial populations inside plants.

Endophytic bacteria live in plant tissues without causing substantive harm to the host. They exist within the living tissues of most plant species in the form of symbiotic association to slightly pathogenic. These bacteria have been isolated from a variety of plants including wheat (Coombs and Franco 2003a, Jha and Kumar 2009, Verma et al 2013, 2014, 2015), rice (Mano and Morisaki 2007, Naik et al 2009, Piromyou et al 2015), mustard (Sheng et al 2008), sugarcane (Mendes et al 2007), maize (Araújo et al 2000, Montanez et al 2012, Thanh and Diep 2014), chilli (Kang et al 2007), potato (Manter et al 2010, Rado et al 2015), tomato (Hallmann et al 1997), soybean (Hung and Annapurna 2004, Mingma et al 2014), pea (Narula et al 2013, Tariq et al 2014), common bean (Suyal et al 2015), sunflower (Forchetti et al 2010, Ambrosini et al 2012), cotton (Quadt-Hallmann et al 1997), chickpea (Saini et al 2015) and strawberry (Hardoim et al 2012). A large number of endophytic bacterial species belonging to different genera including *Achromobacter*, *Azoarcus*, *Burkholderia*, *Enterobacter*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella*, *Microbiospora*, *Micromomospora*, *Nocardioides*, *Pantoea*, *Planomonospora*, *Pseudomonas*, *Serratia*, S*treptomyces* and *Thermomonospora* etc. (Mcinroy and Kloepper 1995, Hallmann et al 1997, Rosenblueth and Martínez-Romero 2006, Ryan et al 2008, Pageni et al 2013, Verma et al 2014, 2015, Mercado-Blanco 2015) have been isolated from different host plants.

These microbes may promote plant growth in terms of increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, roots and shoot length, yield and tolerance to abiotic stresses like drought, temperature, flood, salinity etc. Endophytic microbes can promote plant growth directly through biological nitrogen fixation (BNF); phytohormone production (Indole acetic acid and Gibberellic acids); solubilization of phosphorus (P), potassium (K) and zinc (Zn); inhibition of ethylene biosynthesis in response to biotic or abiotic stress (induced systemic tolerance) etc., or indirectly through inducing resistance to pathogen by production of ammonia, hydrogen cyanide and siderophores (Hallmann et al 1997, Rosenblueth and Martínez-Romero 2006, Verma et al 2015). Biological nitrogen fixation is the process of conversion of atmospheric nitrogen into ammonia.The process of BNF is mostly done by nitrogen fixing microbes. In the recent

agriculture system, much attention has been paid on the biological nitrogen fixation by several endophytic diazotrophic bacteria, such as, *Acetobacter*, *Azoarcus*, and *Herbaspirillum* in gramineous plants. They are mostly found within the plant tissues and contribute to significant nitrogen fixation (James and Olivares 1998, Reinhold-Hurek and Hurek 1998). More than 60% of the nitrogen uptake (about 150 kg N ha/year) from BNF, in Sugarcane cultivars was demonstrated with N^{15} isotope and N balance studies (Urquiaga et al 1992). *Acetobacter, Azoarcus, Azospirillum, Azotobacter, Burkholderia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas have* been reported as BNF in different crops including wheat, barley, maize, sugarcane etc. (Hartmann et al 1995, Baldani et al 1996, Verma et al 2013, Suman et al 2016, Verma et al 2016)

Looking at the importance of endophytic bacteria which increase the crop yield of cereal crops by biological nitrogen fixation and others plant growth promoting attributes, the present investigation would be carried out with the following objectives:

1. Isolation and characterization of endophytic microbes from cereal crops
2. Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes
3. Molecular characterization of selected endophytic microbes
4. Evaluation and validation of selected endophytic microbes for growth and yield of crops

The rationale of the proposed study is to utilize efficient and effective endophytic microbes isolates for developing bio-fertilizers to increase the yield of cereal crop plants. The great importance of the nitrogen resides in the fact that it is an essential constituent of the proteins, nucleic acids and other compounds of nitrogen base essential to the vital processes of all live beings. In this regards, targeting endophytic microbes, a specific group of microbes colonizing cereal crops, could be even more beneficial to be used in the case of different crops, if they possess desired PGP attributes. Therefore, the study of diversity, potential and mechanism of plant growth promotion by endophytic microbes is of immense significance and contribution towards sustainable agriculture

II. REVIEW OF LITERATURE

The endophytic microbe has been reported from different types such as archaea, eubacteria and fungi. Among bacteria, endophytic bacteria were isolated from different phylum mainly: Actinbacteria, Firmicutes, proteobacteria, Bacteroidetes and Deinococcus-Thermus. The Proteobacteria were further grouped as α β and γ-proteobacteria. Distribution of endophytic bacteria varied in all bacterial phyla, Proteobacteria were the most dominant followed by actinobacteria. Least number of endophytic bacteria was reported from phylum Deinococcus-Thermus and Acidobacteria followed by Bacteroidetes, such bacteria were include both Gram-positive and Gram-negative bacteria (Lodewyckx et al 2002, Yadav et al 2015). Endophytic microbes were reported as by both culture-dependent and culture in-dependent approaches. It is possible to assess only a small fraction of the bacterial diversity associated with plants using the isolation methods described above because only a few endophytic bacterial species can be cultivated using traditional laboratory methods. The sizes of bacterial communities as determined using culture-independent methods might be 100- to 1,000-fold larger than communities uncovered via traditional isolation (Yashiro et al 2011).

Endophytic bacteria have been reported in almost every plant studied (Ryan et al 2008). On review of different plants, it was found that endophytic microbes were the most predominant and the studied belonged to three major phyla Actinobacteria, Proteobacteria and Firmicutes. Among 116 reported genera from seventeen different plants (Suman et al 2016), twenty three microbes were reported as the most predominant namely *Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevundimonas, Burkholderia, Cladosporium, Clavibacter, Enterobacter, Flavobacterium, Hannaella, Herbaspirillum, Klebsiella, Methylobacterium, Microbacterium, Microbispora, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Rhodococcus, Sphingomonas, Staphylococcus, Stenotrophomonas* and *Streptomyces* (Mcinroy and Kloepper 1995, Hallmann et al 1997, Reinhold-Hurek and Hurek 1998, Rosenblueth and Martínez-Romero 2006, Ryan et al 2008, Pageni et al 2013, Verma et al 2014, 2015, Mercado-Blanco 2015). Among twenty three genera most predominant were *Bacillus, Burkholderia, Enterobacter, Methylobacterium Pantoea, Pseudomonas* and *Streptomyces* were most dominant and reported on more than five host plants. There were many endophytic bacteria found to be common in more than three host plants. Along with common endophytic microbial genera, many niche specific microbial genera have been reported from all seventeen host plants such as *Mycobacterium, Planobispora, Planomonospora* and *Thermomonospora* from wheat; *Aeromonas, Alkanindiges, Caulobacter, Chlamydomyces, Chryseobacterium, Comamonas,*

Coniothyrium, Gallionella, Geobacter, and *Trichodermaviridae* from rice; *Bradyrhizobium* and *Raoultella* from sugarcane; *Azoarcus*, *Haloferax*, *Methanospirillum*, *Rhanella*, and *Thermoplasma* from maize; *Curtobacterium* and *Guignardia* from citrus; *Frateuria, Janthinobacterium, Ralstonia* and *Sporosarcina* from chilli; *Coryneform frigoribacterium* and *Variovorax* from potato; *Candida* from tomato; *Alternaria, Drechslera, Leuconostoc,* and *Tsukamurella* from soybean; *Mesorhizobium* from chickpea; *Lactobacillus* and *Sphingopyxis* from strawberry (Suman et al 2016).

Most studies on the occurrence of endophytic microbes have been performed using culture-dependent approaches. The genus *Bacillus* has been consistently described as culturable and endophytic, and these bacteria can colonize rice (Sun et al 2008), mustard (Sheng et al 2008), chilli (Rasche et al 2006), citrus (Araújo et al 2001), potato (Sessitsch et al 2004), soybean (Hung and Annapurna 2004), bean (Figueiredo et al 2008), chickpea (Saini et al 2015) and strawberry (Dias et al 2009). The member *Bacillus* and *Bacillus* derived genera (BBDG) associated with different plant show different plant growth promoting attributes such as solubilization of P, K and Zn; production of phytohormones and biocontrol against different pathogens (Tilak et al 2005, Yadav et al 2015). The genus *Burkholderia* has been reported as endophytic in different plant, but most dominant in sugarcane and associated mainly for nitrogen fixation (Suman et al 2001, Castro-González et al 2011). Additionally, other studies have described the importance of members of the genus *Burkholderia* in the cultivation of rice (Govindarajan et al 2008, Rangjaroen et al 2014), maize (Bevivino et al 1998), citrus (Araújo et al 2002) and cotton (Quadt-Hallmann et al 1997). The member of *Enterobacter* bacteria have been reported as endophytic bacteria in different plant such as rice (Piromyou et al 2015), maize (Montanez et al 2012), citrus (Araújo et al 2002), pea (Tariq et al 2014), and strawberry (de Melo Pereira et al 2012). The pink-pigmented facultative methylotrophs (PPFMs) have been reported from diverse host plants including wheat (Yadav et al 2015), rice, citrus, (Dourado et al 2015), bean (de Oliveira Costa et al 2012). In plant colonization, the frequency and distribution may be influenced by plant genotype or by interactions with other associated microorganisms, which may result in increasing plant fitness. The *Methylobacterium* have potential capacity to fix nitrogen, nodule the host plant, produce cytokinins, auxin and enzymes involved in the induction of systemic resistance, such as pectinase and cellulase, and therefore plant growth promotion. The different species of *Pantoea* have been described as cosmopolitan endophytes found in wheat (Verma et al 2014), rice (Rangjaroen et al 2014), sugarcane (Muangthong et al 2015), maize (Ikeda et al 2013), citrus (Araújo et al 2002), chilli (Kang et al 2007) and potato (Reiter et al

2002). Members of *Pantoea* are ubiquitous in plant tissues; they are able to influence plant growth through the production of auxins or cytokinins and induce systemic resistance against diseases. The *Pseudomonas* are ubiquitous in nature, and the member of γ-proteobacteria have been reported from different plant tissues such as wheat, rice (Sun et al 2008), sugarcane (Suman et al 2001), maize (Thanh and Diep 2014, Szilagyi-Zecchin et al 2014), chilli (Kang et al 2007), tomato (Kumar et al 2011), potato (Reiter et al 2003, Sessitsch et al 2004), pearl millet (Gupta et al 2013) and strawberry (de Melo Pereira et al 2012). *Streptomyces* has been reported from shoot, root and seeds of different plant such as wheat (Coombs and Franco 2003a, b), rice (Tian et al 2004), maize (Araújo et al 2000) and chilli (Rasche et al 2006).

Endophytic microbes are of agriculturally important as they can enhance plant growth; improve plant nutrition through nitrogen fixation and other mechanisms. (Araújo et al 2000, Lodewyckx et al 2002, Sun et al 2008, Yanni et al 2011, Pavlo et al 2011, Rangjaroen et al 2014, Verma et al 2015). Endophytes may increase crop yields, remove contaminants, inhibit pathogens, and fixed nitrogen or novel substances (Quadt-Hallmann et al 1997). In endophytic relationships, growth promoting microbes reside within the apoplastic spaces in the host plants. There is direct evidence for the existence of endophytes in the apoplastic intercellular spaces of parenchymal tissue (Dong et al 1997) and the xylem vessels (James et al 1994, Lacava and Azevedo 2013, Glick 2015). Endophyte-infected plants often grow faster than the non infected ones (Cheplick et al 1989). The growth stimulation by endophytes can be a consequence of nitrogen fixation (de Bruijn et al 1997, Suman et al 2001, Iniguez et al 2004, Taulé et al 2012, Pankievicz et al 2015), production of phytohormones, such as IAA and cytokines (Rashid et al 2012, Nath et al 2013, Lin and Xu 2013, Jasim et al 2014, Yadav et al 2015), biocontrol of phytopathogens through the production of antifungal or antibacterial agents, siderophore production, nutrient competition and induction of acquired host resistance, or enhancing the bioavailability of minerals. Several studies have indicated that endophytic colonization can also result in increased plant vigor, and it confers tolerance to biotic and abiotic stresses, enhanced drought tolerance, and improved phosphorus utilization (Yadav et al 2015). Although the interaction between endophytic microbes and their host plants is not fully understood, many isolates showed beneficial effects on their hosts and may play an important role in the physiology of these plants. Several bacterial endophytes have been reported to support plant growth by providing phytohormones, low- molecular weight compounds, or enzymes.

Sustainable agriculture requires the use of strategies to increase or maintain the current rate of food production while reducing damage to the environment and human health. The use of microbial plant growth promoters is an alternative to conventional agricultural technologies. Plant growth-promoting microbe can affect plant growth directly or indirectly. The direct promotion of plant growth by PGPM, for the most part, entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. The indirect promotion of plant growth occurs when PGPM decrease or prevent the deleterious effects of one or more phytopathogenic organisms.

Nitrogen is the major limiting factor for plant growth. The application of N_2-fixing endophytic bacteria as biofertilizer has emerged as one of the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants. Biological nitrogen fixation (BNF) is one of the possible biological alternatives to N-fertilizers and could lead to more productive and sustainable agriculture without harming the environment. Many associative and endophytic bacteria are now known to fix atmospheric nitrogen and supply it to the associated host plants. A variety of nitrogen fixing bacteria like *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, and *Serratia* have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants (James et al 1994, Olivares et al 1996, Giller 2001, Elbeltagy et al 2001, Boddey et al 2003, Wei et al 2014, Reis and Teixeira 2015, White Jr et al 2014).

In recent years, application of endophytic microbial inoculants supplying N requirement efficiently to the various host plants including cereal crops have drawn attention for increasing plant yield in sustainable manner. In terms of benefiting through nitrogen fixation, endophytic microbes are considered to be better than that of rhizospheric one as they provide fixed nitrogen directly to their host plant and fix nitrogen more efficiently due to lower oxygen pressure in the interior of plants than that of soil. The concept of BNF by endophytes (Dobereiner 1992), has led to investigations on the potential uses of endophytic nitrogen-fixing bacteria that colonize graminaceous plants. *Gluconoacetobacter diazotrophicus* is the main contributor of endophytic BNF in sugarcane, which according to nitrogen balance studies fix as high as 150 Kg N $ha^{-1}yr^{-1}$ (Muthukumarasamy et al 2005).

Studies of endophytes in sugarcane have focused on isolation and characterization using morphological and physiological studies of diazotrophic bacteria as well as molecular characterization of *nif* genes and 16S rRNA sequence analysis. Magnani et al 2010, reported that endophytic bacteria live inside sugarcane plant tissues without causing disease; these

bacteria belonged to five groups, based on the 16S rRNA sequences. Group I comprised 14 representatives of the Enterobacteriaceae; group II was composed of Bacilli; group III contained one representative, *Curtobacterium* sp; group IV contained representatives of the Pseudomonadaceae family, and group V had one isolate with an uncultured bacterium. Four isolates were able to reduce acetylene to ethylene. Most of the bacteria isolated from the sugarcane stem and leaf tissues belonged to Enterobacteriaceae and Pseudomonaceae, respectively, demonstrating niche specificity.

Quecine et al (2012), reported that sugarcane growth promotion by the endophytic bacterium *Pantoea agglomerans* 33.1 showing nitrogen fixation. Tam and Diep (2014), isolated and characterized 27 isolates on LGI medium from sugarcane, and all of them have ability of nitrogen fixation and P solubilization together with IAA biosynthesis but there were 10 isolates having the best characteristics. All the endophytic bacteria belonged to Proteobacteria and 3 isolates belonged to Alpha-proteobacteria (30%), 2 isolates were Beta-proteobacteria (20%) 5 isolates were Gamma-proteobacteria (50%). *Enterobacter oryzae* LT7, *Achromobacter xylosoxidans* T16, *Achromobacter insolitus* R15b and *Pantoea agglomerans* T12 revealed promising candidates with multiple beneficial characteristics and they have the potential for application as inoculants or bio-fertilizer adapted to poor latosols and acrisols because they are not only famous strains but also are safety strains for sustainable agriculture. *Burkholderia*, *Herbaspirillum*, *Azospirillum* and *Rhizobium leguminosarum* are contributor of endophytic BNF in rice (Biswas et al 2000, Baldani and Baldani 2005, Govindarajan et al 2008, Isawa et al 2009, Doty 2011, Estrada et al 2013, Choudhury et al 2014, Aon et al 2015). As all of these assumptions seem to support the conditions for nitrogen fixation by endophytic bacteria, there is still no direct evidence that endophytic bacteria actually are the responsible agents of biological nitrogen fixation. Although some agriculturally important crops, such as rize, wheat and maize contain numerous endophytic bacteria such as *Acetobacter diazotrophicus*, *Herbaspirillum* sp., and *Azospirillum* sp., there is little evidence that these bacteria actually fix N_2 in their host plants (James et al 1994).

Ji et al (2014), have isolated 576 endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars and identified 12 of them as diazotrophic bacteria using a specific primer set of *nif* gene. Through 16S rDNA sequence analysis, *nif* H genes were confirmed in the two species of *Penibacillus*, three species of *Microbacterium*, three *Bacillus* species, and four species of *Klebsiella*. Rice seeds treated with these plant growth-promoting bacteria (PGPB) showed improved plant growth, increased height and dry weight and antagonistic effects

against fungal pathogens. In addition, auxin and siderophore producing ability, and P solubilizing activity were studied for the possible mechanisms of plant growth promotion. The widespread use of synthetic fertilizers has resulted in environmental degradation, a decline in beneficial micro- and macroorganisms, and accumulation of chemical residues in the food system. For sustainable agriculture, the use of biologically derived fertilizers would be ecologically sound and economically viable alternatives. These crop-associated indigenous nitrogen fixers may be agronomically important because they could supply part of the nitrogen that the crop requires. Inoculation with N-fixing endophytic bacterium may represent an alternative to the use of chemical N fertilizers and is associated with decreased production costs as well as a considerable increase in crops production.

Verma et al (2014, 2015), reported psychrotolerant and drought tolerant endophytic bacteria from wheat showing nitrogen fixation activity by different genera of *Arthrobacter, Flavobacterium, Bacillus, Methylobacterium, Providencia, Pseudomonas, Stenotrophomonas* and *Enterobacter*. These bacteria also possess solubilization of P, K, Zn; produced IAA, siderophore, ACC deaminase, HCN and ammonia and showed antifungal activity against plant pathogens. Verma et al (2016), have isolated and characterized a total 264 bacteria associated with wheat, which have been isolates from peninsular zone of India, among all isolated bacteria 26 were identified as endophytic of which thirteen diverse groups of bacteria were characterized for nitrogen fixation, such as *Achrommobacter, Alkaligens, Bacillus, Delftia, Providencia, Pseudomonas, Rhodobacter* and *Salmonella.* These biological nitrogen fixing bacteria also solubilized P as well as K at high temperatures. Selected nitrogen fixing, P and K-solubilizing bacteria could be effectively used as biofertilizers at place of chemical fertilizers. NPK could be increase soil productivity to improve sustainability of agriculture production.

III. Methodology

1. **Isolation and characterization of endophytic microbes from cereal crops**
   - Survey and collection of samples from cereal crops (Wheat, Maize, Barley and Oats) growing in Sirmaur district, Himachal Pradesh.
   - Isolation of endophytic microbes using different growth media such as: Nutrient agar medium, King's B agar, Luria-Bertani agar, Jensen agar, Nitrogen free bromothymol Medium (NFB), Tryptic soy agar, Potato dextrose agar, Rose bengal agar etc.
   - Morphological and cultural characterization of isolated microbes on the basis of Size (in mm), Shape (spherical/rod-like/spiral/filamentous/irregular), Colour (Pigmented/ Non-pigmented), Margin (Lobate/Wavy/Smooth), Surface (Smooth/ Rough/Wrinkled) and Polysaccharides producer.

2. **Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes**
   - Nitrogen fixing attributes using Acetylene Reduction Assay
   - Screening of endophytic microbes for other plant growth promoting attributes including P, K and Zn solubilization, production of siderophore and phytohormones

3. **Molecular characterization of endophytic microbes**
   - Isolation of genomic DNA from selected endophytic microbes
   - PCR amplification of 16S rDNA and candidate nitrogen fixation gene (s)
   - Sequencing of 16S rRNA genes and nitrogen fixation gene (s) for identification
   - Phylogenetic analyses will be carried out of 16S rRNA genes using MEGA 6.

4. **Evaluation and validation of selected endophytic microbes for growth and yield of crops**
   - Seed germination bioassay of selected microbial isolates with N_2-fixtion and other PGP attributes
   - Evaluation of endophytic microbes *in vitro* and *in vivo* for PGP and yield of selected crops

IV. Plan of Research Work

Time schedule of activities and activity milestones

S. No.	Objectives	When to be attained					
		1st Year		2nd Year		3rd Year	
		I	II	I	II	I	II
1.	Isolation and characterization of endophytic microbes from cereal crops						
2.	Characterization of endophytic microbes for N_2-fixation and other PGP attributes						
3.	Molecular characterization of selected endophytic microbes						
4.	Evaluation and validation of selected endophytic microbes for growth and yield of crops						

V. WORK DONE

Objective 1: Isolation and characterization of endophytic microbes from cereal crops

- Plant samples from cereal crops (wheat, oat and barley) were collected from Baru sahib, Sirmaur, Himachal Pradesh, India.
- Endophytic microbes has been isolated using thirteen different selective and complex growth media
- A total of **237** microbes have been isolated from wheat (159), Barley (43) and oat (35).
- Isolated microbes have been characterized for their morphological characteristic.

Objective 2: Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes

- All isolates have been screened for plant growth promoting attributes (solubilization of P, K and Zn; production of ammonia, siderophores, hydrogen cyanide and indole acetic acid).

VI. EXPECTED OUTCOMES OF RESEARCH

- Advancement in taxonomy of endophytic microbes
- Mapping of extent of total genetic variability of endophytic microbial isolates.
- Identification of novel species/strain of endophytic microbes
- Development of N_2-fixing bio- inoculants for cereal crops

References

Ambrosini A, Beneduzi A, Stefanski T, Pinheiro F, Vargas L, Passaglia LP (2012) Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). *Plant Soil* **356**: 245-264.

Aon M, Khalid M, Hussain S, Naveed M, Akhtar MJ (2015) diazotrophic inoculation supplemented nitrogen demand of flooded rice under field conditions. *Pak J Agri Sci.* **52:**145-150.

Araújo JMd, Silva ACd, Azevedo JL (2000) Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea mays* L.). *Braz Arch Biol Technol.* doi:10.1590/S1516-89132000000400016

Araújo WL, Maccheroni Jr W, Aguilar-Vildoso CI, Barroso PA, Saridakis HO, Azevedo JL (2001) Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Can J Microbiol.* **47**: 229-236.

Araújo WL, Marcon J, Maccheroni W, van Elsas JD, van Vuurde JW, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants. *Appl Environ Microbiol.* **68**: 4906-4914.

Baldani J, Pot B, Kirchhof G, Falsen E, Baldani V, Olivares F, Hoste B, Kersters K, Hartmann A, Gillis M (1996) Emended Description of *Herbaspirillum*; Inccusion of [*Pseudomonas*] *rubrisubalbicans*, a Mild Plant Pathogen, as *Herbaspirillum rubrisubalbicans* comb. Nov and Classification of a Group of Clinical Isolates (EF Group 1) as *Herbaspirillum* Species 3. *Int J Sys Evol Microbiol.* **46**: 802-810.

Baldani JI, Baldani VL (2005) History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience. *Anais da Academia Brasileira de Ciências* **77**: 549-579.

Bell C, Dickie G, Harvey W, Chan J (1995) Endophytic bacteria in grapevine. *Can J Microbiol.* **41**: 46-53.

Bevivino A, Sarrocco S, Dalmastri C, Tabacchioni S, Cantale C, Chiarini L (1998) Characterization of a free-living maize-rhizosphere population of *Burkholderia cepacia*: effect of seed treatment on disease suppression and growth promotion of maize. *FEMS Microbiol Ecol.* **27**: 225-237.

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG (2000) Rhizobial inoculation influences seedling vigor and yield of rice. *Agron J.* **92**: 880-886.

Boddey RM, Urquiaga S, Alves BJ, Reis V (2003) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. *Plant Soil* **252**:139-149.

Castro-González R, Martínez-Aguilar L, Ramírez-Trujillo A, Estrada-de los Santos P, Caballero-Mellado J (2011) High diversity of culturable *Burkholderia* species associated with sugarcane. *Plant Soil* **345**:155-169.

Cheplick G, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. *New Phytol.* **111**: 89-97.

Choudhury AT, Kecskés ML, Kennedy IR (2014) Utilization of BNF Technology Supplementing Urea N for Sustainable Rice Production. *J Plant Nutr.* **37**: 1627-1647.

Coombs JT, Franco CM (2003a) Isolation and identification of Actinobacteria from surface-sterilized wheat roots. *Appl Environ Microbiol.* **69**: 5603-5608.

Coombs JT, Franco CM (2003b) Visualization of an endophytic *Streptomyces* species in wheat seed. *Appli Environ Microbiol.* **69**: 4260-4262.

de Bruijn F, Stoltzfus J, So R, Malarvithi P, Ladha J (1997) Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. In: Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes. Springer, pp 25-36.

de Melo Pereira G, Magalhães K, Lorenzetii E, Souza T, Schwan R (2012) A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. *Microb Ecol.* **63**: 405-417

de Oliveira Costa LE, de Queiroz MV, Borges AC, de Moraes CA, de Araújo EF (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). *Braz J Microbiol.* **43**: 1562.

Dias AC, Costa FE, Andreote FD, Lacava PT, Teixeira MA, Assumpção LC, Araújo WL, Azevedo JL, Melo IS (2009) Isolation of micropropagated strawberry endophytic

bacteria and assessment of their potential for plant growth promotion. *World J Microbiol Biotechnol.* **25**: 189-195.

Dobereiner J (1992) History and new perspectives of diazotrophs in association with non-leguminous plants. *Symbiosis* 3: 1-13.

Dong Z, McCully M, Canny M (1997) Does *Acetobacter diazotrophicus* live and move in the xylem of sugarcane stems? Anatomical and physiological data. *Ann Bot.* **80**: 147-158.

Doty SL (2011) Nitrogen-fixing endophytic bacteria for improved plant growth. In: Bacteria in Agrobiology: Plant Growth Responses. Springer, pp 183-199.

Dourado MN, Aparecida Camargo Neves A, Santos DS, Araújo WL (2015) Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. *BioMed Res Int.* doi:10.1155/2015/909016

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, Isawa T, Mitsui H, Minamisawa K (2001) Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. *Appl Environ Microbiol.* **67**: 5285-5293.

Estrada GA, Baldani VLD, de Oliveira DM, Urquiaga S, Baldani JI (2013) Selection of phosphate-solubilizing diazotrophic *Herbaspirillum* and *Burkholderia* strains and their effect on rice crop yield and nutrient uptake. *Plant Soil* **369**: 115-129.

Feller IC (1995) Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). *Ecol Monogr.* **64**: 477-505.

Figueiredo M, Martinez C, Burity H, Chanway C (2008) Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). *World J Microbiol Biotechnol.* **24**: 1187-1193.

Forchetti G, Masciarelli O, Izaguirre MJ, Alemano S, Alvarez D, Abdala G (2010) Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. *Curr Microbiol.* **61**: 485-493.

Giller KE (2001) Nitrogen fixation in tropical cropping systems. Oxon, GB: CAB International.

Glick BR (2015) Introduction to Plant Growth-promoting Bacteria. In: Beneficial Plant-Bacterial Interactions. Springer, pp 1-28.

Govindarajan M, Balandreau J, Kwon S-W, Weon H-Y, Lakshminarasimhan C (2008) Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. *Microb Ecol.* **55**: 21-37.

Gupta G, Panwar J, Jha PN (2013) Natural occurrence of *Pseudomonas aeruginosa*, a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br. *Appl Soil Ecol.* **64**: 252-261.

Hallmann J, Quadt-Hallmann A, Mahaffee W, Kloepper J (1997) Bacterial endophytes in agricultural crops. *Can J Microbiol.* **43**: 895-914.

Hardoim P, Nissinen R, van Elsas JD (2012) Ecology of bacterial endophytes in sustainable agriculture. In: Bacteria in Agrobiology: Plant Probiotics. Springer, pp 97-126.

Hartmann A, Baldani JI, Kirchhof G, Aßmus B, Hutzler P, Springer N, Ludwig W, Baldani VLD, Döbereiner J (1995) Taxonomic and ecologic studies of diazotrophic rhizosphere bacteria using phylogenetic probes. In: *Azospirillum* VI and Related Microorganisms. Springer, pp 415-427.

Hung PQ, Annapurna K (2004) Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.). *Omonrice* **12**: 92-101.

Ikeda AC, Bassani LL, Adamoski D, Stringari D, Cordeiro VK, Glienke C, Steffens MBR, Hungria M, Galli-Terasawa LV (2013) Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. *Microb Ecol.* **65**: 154-160.

Iniguez AL, Dong Y, Triplett EW (2004) Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. *Mol Plant Microbe In.* **17**: 1078-1085.

Isawa T, Yasuda M, Awazaki H, Minamisawa K, Shinozaki S, Nakashita H (2009) *Azospirillum sp.* strain B510 enhances rice growth and yield. *Microbes Environ.* **25**: 58-61.

Jacobs MJ, Bugbee WM, Gabrielson DA (1985) Enumeration, location, and characterization of endophytic bacteria within sugar beet roots. *Can J Bot.* **63**: 1262-1265.

James E, Reis V, Olivares F, Baldani J, Döbereiner J (1994) Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. *J Exp Bot.* **45**: 757-766.

James EK, Olivares FL (1998) Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Cri Rev Plant Sci.* **17**: 77-119.

Jasim B, Jimtha John C, Shimil V, Jyothis M, Radhakrishnan E (2014) Studies on the factors modulating indole-3-acetic acid production in endophytic bacterial isolates from *Piper nigrum* and molecular analysis of ipdc gene. *J Appl Microbiol.* **117**: 786-799.

Jha P, Kumar A (2009) Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microb Ecol.* **58**: 179-188.

Ji SH, Gururani MA, Chun S-C (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol Res.* **169**: 83-98.

Kang SH, Cho H, Cheong H, Ryu C, Kim JF, Park S (2007) Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum L.*). *J Microbiol Biotechnol.* **17**: 96-103.

Kluepfel DA (1993) The behavior and tracking of bacteria in the rhizosphere. *Annu Rev Phytopathol.* **31**: 441-472.

Kobayashi D, Palumbo J (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Microbial endophytes. pp. 199-233.

Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC (2011) Isolation and characterization of rhizobacteria associated with coastal agricultural ecosystem of rhizosphere soils of cultivated vegetable crops. *World J Microbiol Biotechnol.* **27**: 1625-1632.

Lacava PT, Azevedo JL (2013) Endophytic bacteria: a biotechnological potential in agrobiology system. In: Bacteria in Agrobiology: Crop Productivity. Springer, pp 1-44.

Lin L, Xu X (2013) Indole-3-acetic acid production by endophytic *Streptomyces* sp. En-1 isolated from medicinal plants. *Curr Microbiol.* **67**: 209-217.

Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie Dv (2002) Endophytic bacteria and their potential applications. *Cri Rev Plant Sci*. **21**: 583-606.

Magnani G, Didonet C, Cruz L, Picheth C, Pedrosa F, Souza E (2010) Diversity of endophytic bacteria in Brazilian sugarcane.*Genet Mol Res*. **9**: 250-258.

Mano H, Morisaki H (2007) Endophytic bacteria in the rice plant. *Microbes Environ*. **23**: 109-117.

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. *Microb Ecol.* **60**: 157-166.

Marler M, Pedersen D, Mitchell-Olds T, Callaway R (1999) A polymerase chain reaction method for detecting dwarf mistletoe infection in Douglas-fir and western larch. *Can J Forest Res*. **29**: 1317-1321.

Mcinroy JA, Kloepper JW (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil*. **173**: 337-342.

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. *Appl Environ Microbiol*. **73**: 7259-7267.

Mercado-Blanco J (2015) Life of Microbes Inside the Plant. In: Principles of Plant-Microbe Interactions. Springer, pp 25-32.

Mercado-Blanco J, JJ Lugtenberg B (2014) Biotechnological applications of bacterial endophytes. *Curr Biotechnol*. **3**: 60-75.

Mingma R, Pathom-aree W, Trakulnaleamsai S, Thamchaipenet A, Duangmal K (2014) Isolation of rhizospheric and roots endophytic actinomycetes from Leguminosae plant and their activities to inhibit soybean pathogen, *Xanthomonas campestris* pv. *glycine*. *World J Microbiol Biotechnol*. **30**: 271-280.

Montanez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. *Appl Soil Ecol.* **58**: 21-28.

Muangthong A, Youpensuk S, Rerkasem B (2015) Isolation and characterization of endophytic nitrogen fixing bacteria in sugarcane. *Trop Life Sci Res*. **26:** 41-51.

Muthukumarasamy R, Cleenwerck I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Park K-D, Son CY, Sa T (2005) Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. *Syst Appl Microbiol*. **28**: 277-286.

Naik BS, Shashikala J, Krishnamurthy Y (2009) Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities *in vitro*. *Microbiol Res*. **164**: 290-296.

Narula S, Anand R, Dudeja S, Pathak D (2013) Molecular diversity of root and nodule endophytic bacteria from field pea (*Pisum sativum* L.). *Leg Res.* **36**: 344-350.

Nath R, Sharma G, Barooah M (2013) Screening of endophytic bacterial isolates of Tea (*Camellia sinensis* L.) roots for their multiple plant growth promoting activities. *Int J Agri Environ Biotech*. **6**: 211-215.

Olivares FL, Baldani VL, Reis VM, Baldani JI, Döbereiner J (1996) Occurrence of the endophytic diazotrophs *Herbaspirillum* sp. in roots, stems, and leaves, predominantly of Gramineae. *Bio Fert Soils* **21**: 197-200

Pageni BB, Lupwayi NZ, Larney FJ, Kawchuk LM, Gan Y (2013) Populations, diversity and identities of bacterial endophytes in potato (*Solanum tuberosum* L.) cropping systems. *Can J Plant Sci*. **93**: 1125-1142.

Pankievicz V, Amaral FP, Santos KF, Agtuca B, Xu Y, Schueller MJ, Arisi ACM, Steffens M, Souza EM, Pedrosa FO (2015) Robust biological nitrogen fixation in a model grass–bacterial association. *Plant J.* **81**: 907-919.

Patriquin D, Döbereiner J (1978) Light microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. *Can J Microbiol.* **24**: 734-742.

Pavlo A, Leonid O, Iryna Z, Natalia K, Maria PA (2011) Endophytic bacteria enhancing growth and disease resistance of potato (*Solanum tuberosum* L.). *Biol Control*. **56**: 43-49.

Piromyou P, Greetatorn T, Teamtisong K, Okubo T, Shinoda R, Nuntakij A, Tittabutr P, Boonkerd N, Minamisawa K, Teaumroong N (2015) Preferential Association of Endophytic *Bradyrhizobia* with Different Rice Cultivars and Its Implications for Rice Endophyte Evolution. *Appl Environ Microbiol.* **81**: 3049-3061.

Quadt-Hallmann A, Kloepper J, Benhamou N (1997) Bacterial endophytes in cotton: mechanisms of entering the plant. *Can J Microbiol*. **43**: 577-582.

Quecine M, Araújo W, Rossetto P, Ferreira A, Tsui S, Lacava P, Mondin M, Azevedo J, Pizzirani-Kleiner A (2012) Sugarcane growth promotion by the endophytic bacterium *Pantoea agglomerans* 33.1. *Appl Environ Microbiol.* **78**: 7511-7518.

Rado R, Andrianarisoa B, Ravelomanantsoa S, Rakotoarimanga N, Rahetlah V, Fienena F, Andriambeloson O (2015) Biocontrol of potato wilt by selective rhizospheric and endophytic bacteria associated with potato plant. *Afr J Food Agric Nutr Dev.* **15**: 9762-9776.

Rangjaroen C, Rerkasem B, Teaumroong N, Noisangiam R, Lumyong S (2014) Promoting plant growth in a commercial rice cultivar by endophytic diazotrophic bacteria isolated from rice landraces. *Ann Microbiol.* **65**: 253-266

Rasche F, Trondl R, Naglreiter C, Reichenauer TG, Sessitsch A (2006) Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum anuum* L.). *Can J Microbiol.* **52**: 1036-1045.

Rashid S, Charles TC, Glick BR (2012) Isolation and characterization of new plant growth-promoting bacterial endophytes. *Appl Soil Ecol*. **61**: 217-224.

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. *Trends Microbiol.* **6**: 139-144.

Reis VM, Teixeira KRS (2015) Nitrogen fixing bacteria in the family Acetobacteraceae and their role in agriculture. *J Basic Microbiol.* **55**: 931-949.

Reiter B, Pfeifer U, Schwab H, Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl Environ Microbiol.* **68**: 2261-2268.

Reiter B, Wermbter N, Gyamfi S, Schwab H, Sessitsch A (2003) Endophytic *Pseudomonas* spp. populations of pathogen-infected potato plants analysed by 16S rDNA and 16S rRNA-based denaturating gradient gel electrophoresis. *Plant Soil* **257**: 397-405.

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. *Mol Plant Microb IN*. **19**: 827-837.

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett*. **278**: 1-9.

Saini R, Dudeja SS, Giri R, Kumar V (2015) Isolation, characterization, and evaluation of bacterial root and nodule endophytes from chickpea cultivated in Northern India. *J Basic Microbiol*. **55**: 74-81.

Schulz B, Boyle C (2005) The endophytic continuum. *Mycol Res*. **109**: 661-686.

Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. *Can J Microbiol.* **50**: 239-249.

Sheng X-F, Xia J-J, Jiang C-Y, He L-Y, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ Poll*. **156**:1164-1170.

Stone JK, Bacon CW, White J (2000) An overview of endophytic microbes: endophytism defined. In: Microbial endophytes, pp 29-33.

Suman A, Shasany AK, Singh M, Shahi HN, Gaur A, Khanuja SPS (2001) Molecular assessment of diversity among endophytic diazotrophs isolated from subtropical Indian sugarcane. *World J Microbiol Biotechnol*. **17**: 39-45.

Suman A, Yadav AN, Verma P (2016) Endophytic microbes in crops: Diversity and Beneficial impact for Sustainable Agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity, Vol.1, Research Perspectives, pp117-143.

Sun L, Qiu F, Zhang X, Dai X, Dong X, Song W (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microb Ecol*. **55**: 415-424.

Suyal DC, Yadav A, Shouche Y, Goel R (2015) Bacterial diversity and community structure of Western Indian Himalayan red kidney bean (*Phaseolus vulgaris*) rhizosphere as revealed by 16S rRNA gene sequences. *Biologia* **70**: 305-313.

Szilagyi-Zecchin VJ, Ikeda AC, Hungria M, Adamoski D, Kava-Cordeiro V, Glienke C, Galli-Terasawa LV (2014) Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. *AMB Express* 4:26.

Tam HM, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in sugarcane (*Saccharum* spp. L.*)* cultivated on soils of the Dong Nai province, Southeast of Vietnam. *Am J Life Sci.* **2**: 361-368.

Tariq M, Hameed S, Yasmeen T, Zahid M, Zafar M (2014) Molecular characterization and identification of plant growth promoting endophytic bacteria isolated from the root nodules of pea (*Pisum sativum* L.). *World J Microbiol Biotechnol*. **30**: 719-725.

Taulé C, Mareque C, Barlocco C, Hackembruch F, Reis VM, Sicardi M, Battistoni F (2012) The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. *Plant Soil* **356**: 35-49.

Thanh DTN, Diep CN (2014) Isolation, characterization and identification of endophytic bacteria in maize (*Zea mays* L.) cultivated on Acrisols of the Southeast of Vietnam. *Am J Life Sci*. **2**: 224-233.

Tian X, Cao L, Tan H, Zeng Q, Jia Y, Han W, Zhou S (2004) Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. *World J Microbiol Biotechnol*. **20**: 303-309.

Tilak K, Ranganayaki N, Pal K, De R, Saxena A, Nautiyal CS, Mittal S, Tripathi A, Johri B (2005) Diversity of plant growth and soil health supporting bacteria. *Curr Sci*. **89**: 136-150.

Urquiaga S, Cruz KH, Boddey RM (1992) Contribution of nitrogen fixation to sugar cane: nitrogen-15 and nitrogen-balance estimates. *Soil Sci Am J* . **56**: 105-114.

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2013) Elucidating the diversity and plant growth promoting attributes of wheat (*Triticum aestivum*) associated acidotolerant bacteria from southern hills zone of India. *Natl J Life Sci* .**10**: 219-226.

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2014) Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India. *Int J Curr Microbiol App Sci.* **3**: 432-447.

Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK, Suman A (2016) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. *Saudi J Bio Sci.* doi:10.1016/j.sjbs.2016.01.042

Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK, Suman A (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. *Ann Microbiol*. 65: 1885-1899.

Wei C-Y, Lin L, Luo L-J, Xing Y-X, Hu C-J, Yang L-T, Li Y-R, An Q (2014) Endophytic nitrogen-fixing *Klebsiella variicola* strain DX120E promotes sugarcane growth. *Biol Fert Soils* **50**: 657-666.

White Jr JF, Torres MS, Johnson H, Irizarry I, Tadych M (2014) A functional view of plant microbiomes: endosymbiotic systems that enhance plant growth and survival. In: Advances in Endophytic Research. Springer, pp 425-439.

Yadav AN, Sharma D, Gulati S, Singh S, Dey R, Pal KK, Saxena AK (2015) Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. *Sci Rep.* **5**: 12293.

Yanni Y, Dazzo F, Zidan M (2011) Beneficial Endophytic Rhizobia as Biofertilizer Inoculants for Rice and the Spatial Ecology of This Bacteria–Plant Association. In: Bacteria in Agrobiology: Crop Ecosystems. Springer, pp 265-294.

Yashiro E, Spear R, McManus P (2011) Culture-dependent and culture-independent assessment of bacteria in the apple phyllosphere. *J Appl Microbiol.* **110**: 1284-1296.

ADVISORY COMMITTEE

Name of the Student: **Mrs Kusam Lata Rana**

Reg. No.: **BS15PSBT003**

Department: **Biotechnology**

College: **Agriculture**

Major Advisor: **Dr. Ajar Nath Yadav**

S. No.	Name and Department	Role	Signature
1.	Dr. Ajar Nath Yadav, Biotechnology	Major Advisor	
2.	Dr. Vinod Kumar, Biotechnology	Co-Major Advisor	
3.	Dr. H.S. Dhaliwal, Agriculture	Member	
4.	Dr. Pritesh Vyas, Biotechnology	Member	
5.	Dr. N. Ramu, Agronomy	Member	

HOD 15/11/2016
(Signature)

Major Advisor
(Signature)

Dean PGS
(Signature)

Dean of College
(Signature)

Synopsis approved as such

For Approval

Vice Chancellor

ADVISORY COMMITTEE MEETING

Name of the Student: MRS KUSAM LATA RANA

Major Subject: BIOTECHNOLOGY

Major Advisor: DR. AJAR NATH YADAV

Name of Department: BIOTECHNOLOGY

Name of College: AKAL COLLEGE OF AGRICULTURE

Venue: DEPARTMENT OF BIOTECHNOLOGY **Date: 25-10-2016** **Time: 3:0 pm**

Students Work Load Teaching

S.No.	Course No	Title of Course	Credit Hrs
1	MIC-201	Fermentation and Industrial Microbiology	03

Title of Thesis: Nitrogen Fixing Endophytic Microbes from Cereal Crops and their Biotechnological Applications

Research Work Carried out in the present Semester

- Plant samples from cereal crops (wheat, oat and barley) were collected from Baru sahib, Sirmaur, Himachal Pradesh, India.
- A total of 237 endophytic microbes have been isolated using thirteen different selective and complex growth media.
- Isolated microbes have been characterized for their morphological characteristic.
- All isolates have been screened for plant growth promoting attributes (solubilization of phosphorus, potassium and zinc; production of ammonia, siderophores, hydrogen cyanide and indole acetic acid).

Research Work to be carried out in the next semester

- Characterization of microbes for nitrogen fixation attributes
- Molecular characterization of selected endophytic microbes

Kusam

Signature of the Student

Signature of Major Advisor

Committee Members:

1. Dr. Ajar Nath Yadav .....
2. Dr. Vinod Kumar .....
3. Dr. H.S. Dhaliwal .....
4. Dr. Pritesh Vyas .....
5. Dr. N. Ramu .....